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Application Note 01992

Quantitation of Multi-Class Pharmaceuticals in Water Using Simultaneous Positive and Negative ESI on the Varian 320-MS Triple Quadrupole Mass Spectrometer

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Introduction

Reports of pharmaceutical contaminants in waste and surface water began in the 1970s. Since then researchers around the globe have reported trace levels of pharmaceuticals in aquatic environments and links to the disruption of animal proliferation.¹ Additional studies attribute chronic exposure of trace levels of antibiotics in the environment to the emergence of more resistant strains of bacteria.²

With the increasing production and use of pharmaceuticals, there is a growing trend for excretion or improper disposal of these compounds into sewage wastewater treatment plants.

Agencies, such as the US Environmental Protection Agency (US EPA) and the European Organization for Economic Co-operation and Development (OECD), are developing methods to monitor endocrine disrupting chemicals (EDC) in treated wastewater, surface water and drinking water.

The Varian 320-MS triple quadrupole mass spectrometer was used in both positive and negative ESI Multiple Reaction Monitoring (MRM) mode to quantify a broad range of pharmaceuticals. Instrument and calibration results for twelve multi-class pharmaceuticals are presented over a range of 1–50 ng/mL.

Instrumentation

- Varian 320-MS triple quadrupole mass spectrometer with ESI source
- Varian 212-LC binary solvent delivery modules
- Varian ProStar™ 430 AutoSampler

Materials and Reagents

Standards were obtained from Sigma-Aldrich (St. Louis, MO). Optima grade acetonitrile and HPLC grade methanol were obtained from Fisher Scientific (Pittsburgh, PA).

Standard Stock Solutions

Individual stock solutions were prepared in methanol at 1 mg/mL in amber glass vials and stored at -10 °C. Five calibration standards were prepared daily in water at 50, 25, 10, 5 and 1 ng/mL from a 50 µg/mL working stock.

LC Conditions

Column: Pursuit™ XRs C18, 3 µm, 50 x 2.0 mm
Varian Part No. A6001050X020 (column)
A6001MG2 (guard)

Solvent A: 0.1% formic acid

Solvent B: Acetonitrile

| LC Program: | Time (min:sec) | %A | %B | Flow (µL/min) |
|-------------|-------------------|-----|----|------------------|
| | 0:00 | 100 | 0 | 200 |
| | 0:30 | 100 | 0 | 200 |
| | 04:00 | 70 | 30 | 200 |
| | 08:30 | 10 | 90 | 200 |
| | 10:30 | 10 | 90 | 200 |
| | 11:00 | 100 | 0 | 200 |
| | 14:00 | 100 | 0 | 200 |

MS Parameters

Ionization Mode: ESI (positive and negative)

Drying Gas: 25 psi at 310 °C

Nebulizing Gas: 55 psi

Needle: 5000 V

Shield: 600 V

Table 1. MS segment parameters.

| Analyte | MRM | Capillary Voltage (V) | Collision Energy (V) |
|-----------------|---------------|-----------------------|----------------------|
| Amoxicillin | (+) 366 → 114 | 49 | 15 |
| Acetaminophen | (+) 152 → 110 | 49 | 16 |
| Caffeine | (+) 195 → 138 | 45 | 16 |
| Thiabendazole | (+) 202 → 175 | 45 | 22 |
| Trimethoprim | (+) 291 → 123 | 60 | 22 |
| Carbamazepine | (+) 237 → 194 | 50 | 16 |
| Sulfamethoxazol | (+) 254 → 156 | 48 | 12 |
| Diphenhydramin | (+) 256 → 167 | 10 | 10 |
| Salicylic Acid | (-) 137 → 93 | -50 | 16 |
| Warfarin | (+) 309 → 163 | 45 | 12 |
| Ibuprofen | (-) 205 → 161 | -40 | 6 |
| Gemfibrozil | (-) 249 → 121 | -48 | 10 |

Results and Discussion

The automated optimization features of Varian Workstation software quickly identified the conditions for the mass spectrometer as tabulated above.

Since four compounds had better sensitivity in negative ESI mode, simultaneous positive and negative MS/MS scans were collected.

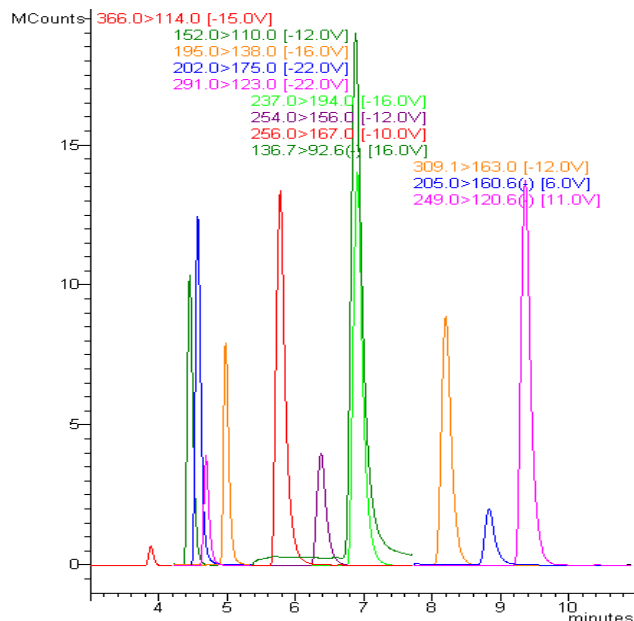


Figure 1. Overlay of MRM chromatograms for all 12 pharmaceuticals.

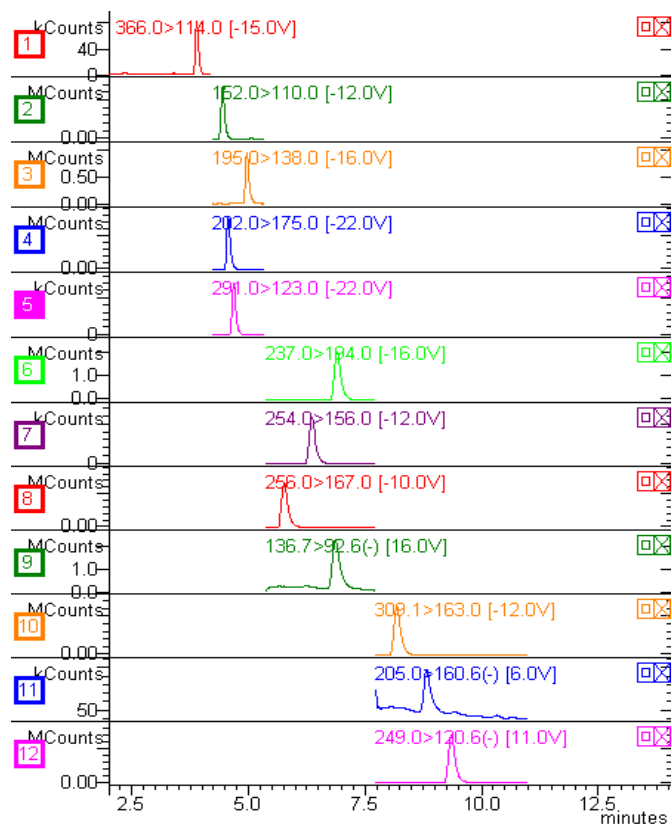


Figure 2. MRM chromatograms for pharmaceuticals at 5 ng/mL.

The Varian Pursuit™ XRs C18 column offers outstanding separation of all 12 pharmaceuticals in less than 10 minutes, as shown in Figure 1.

The 5 ng/mL standard, shown in Figure 2, was injected 12 times with the % relative standard deviation (%RSD) tabulated in Table 2. The %RSD is less than 13% for all compounds and less than 9% for 11 of the 12 compounds.

Table 2. Repeatability for 12 injections at 5 ng/mL.

| Compound | Retention Time (min) | %RSD |
|---------------------|----------------------|------|
| Amoxicillin | 3.87 | 8.7 |
| Acetaminophen | 4.37 | 3.9 |
| Thiabendazole | 4.94 | 3.1 |
| Trimethoprim | 4.56 | 5.7 |
| Caffeine | 4.68 | 2.1 |
| Diphenhydramine HCl | 6.93 | 2.6 |
| Sulfamethoxazole | 6.30 | 6.0 |
| Salicylic Acid | 5.85 | 4.1 |
| Carbamazepine | 6.82 | 7.0 |
| Warfarin | 8.20 | 4.2 |
| Ibuprofen | 8.82 | 12.2 |
| Gemfibrozil | 9.34 | 4.5 |

Calibration standards at 1, 5, 10, 25 and 50 ng/mL were linear with r^2 values greater than 0.995 (Table 3).

Table 3. Calibration data from 1–50 ng/mL.

| Compound | r^2 |
|---------------------|--------|
| Amoxicillin | 0.9997 |
| Acetaminophen | 0.9979 |
| Thiabendazole | 0.9960 |
| Trimethoprim | 0.9957 |
| Caffeine | 0.9985 |
| Diphenhydramine HCl | 0.9993 |
| Sulfamethoxazole | 0.9987 |
| Salicylic Acid | 0.9999 |
| Carbamazepine | 0.9948 |
| Warfarin | 0.9955 |
| Ibuprofen | 0.9997 |
| Gemfibrozil | 0.9975 |

Conclusion

This application demonstrates the ability of the 320-MS to provide rapid analysis and accurate quantitation of a broad range of pharmaceutical compounds in water. Both positive and negative ions are simultaneously acquired with excellent sensitivity, and a run time of less than 10 min was obtained with a standard HPLC system.

References

1. Kim, S; Cho, J; Kim, I; Vanderford, B; Snyder, S; Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters. *Water Research* 41(2007) 1013-1021.
2. Batt, A.; Aga, D. Simultaneous Analysis of Multiple Classes of Antibiotics by Ion Trap LC/MS/MS for Assessing Surface Water and Groundwater Contamination. *Anal. Chem.* 77 (2005) 2940-2947.

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